THE INHERITANCE OF DOUBLENESS IN MATTHIOLA AND PETUNIA. I. THE HYPOTHESES*

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THE peculiar inheritance of "doubleness" in stocks (*Matthiola*) has long been a matter of special interest. Some races produce only single-flowering plants. A pure double-flowering race, on the other hand, is an impossibility; the doubles are absolutely sterile, stamens and pistils

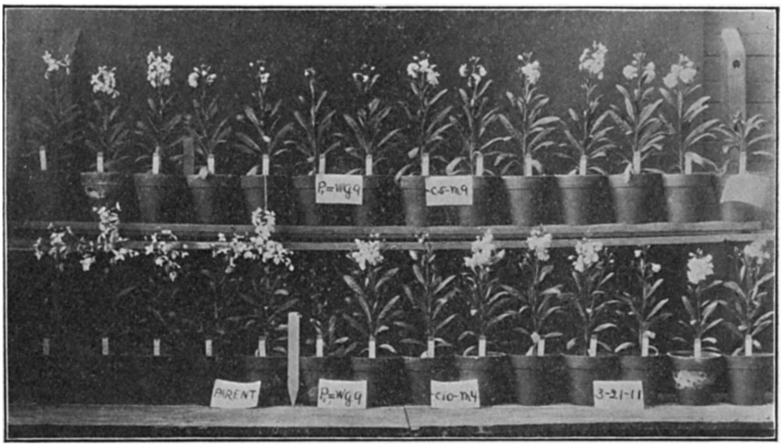

FIG. 1. *Matthiola* plants, unselected progeny of two parents, showing the mixture of singles and doubles invariably given by this variety ("Snowflake"). Note that the singles, in these cool-greenhouse cultures, differ little from the doubles in earliness of flowering. (The singles and doubles have been separated for photographing. The singles are plants 1, 2, 3, and 11 (misplaced), from the left side, in the upper row, and plants 1 to 5 in the lower row.)

being entirely absent. Certain races, however, consist of both singles and doubles, in nearly equal numbers,[1] each generation being descended from the singles of the pre-

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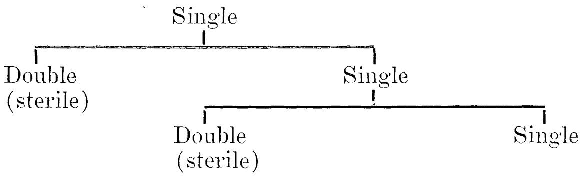
[1] The usual proportion of doubles in large cultures seems to be near 53 per cent., or perhaps slightly higher in some cases.

ceding generation (see Fig. 1); the following diagram shows the mode of inheritance in such races:



Miss Saunders (1911; 1913; Bateson, 1909, pp. 201–204) has done a great amount of work on heredity in *Matthiola*, and has developed an ingenious hypothesis to explain the peculiar behavior of doubleness. Goldschmidt (1913) has given another explanation, which has been vigorously criticized by Miss Saunders. Several years ago (perhaps in 1909), largely on the basis of Miss Saunders's evidence, I formulated a hypothesis somewhat simpler than either of those just mentioned.

In view of the special interest of the case at present, and the fact that one or both of the essential points of my explanation have been suggested incidentally by another writer (Belling, 1915, 1915a), it seems desirable to give a general review of the hypotheses at this time.[2]

As Miss Saunders's (1911; Bateson, 1909, p. 201–204) crosses have shown, the "double-throwing" singles are heterozygous, the approximately 1:1 ratio being due to the fact that the functional pollen is all double-carrying. This is shown by Miss Saunders's crosses between double-throwers and pure singles. When the double-thrower is the seed-parent, about half the F_1 progeny are heterozygous, the rest being pure singles; about half the double-thrower eggs, then, are "double-carrying." On the other

[2] My own data bearing on the problem have largely been published (Frost, 1911) or will be published in two papers (Frost, unpublished) soon to appear; some further evidence, relating to the proportions secured with some 8,000 plants of one variety, together with a summary of my other data, is to be presented in a paper to follow the present one. Aside, however, from one important general feature of these results, to be briefly stated below, the view given here is dependent on Miss Saunders's evidence and that cited by her.

hand, when the double-thrower is the pollen-parent, *all* the F_1 progeny are heterozygous; hence all the double-thrower-pollen is double-carrying. These facts are illustrated by the two following diagrams (adapted from Goldschmidt):

P_1 Double-thrower ♀ —— Pure single ♂

F_1 Single ($\frac{1}{2}$) Single ($\frac{1}{2}$)

F_2 Single Single ($\frac{1}{4}$) Single ($\frac{1}{2}$) Double ($\frac{1}{4}$)

F_3 Single (pure) Single (pure) Single ($\frac{1}{4}$) (pure) Single ($\frac{1}{2}$) (heterozygous) Double ($\frac{1}{4}$) (sterile)

P_1 Pure single ♀ —— Double-thrower ♂

F_1 Single

F_2 Single ($\frac{1}{4}$) Single ($\frac{1}{2}$) Double ($\frac{1}{4}$)

F_3 Single (pure) Single ($\frac{1}{4}$) (pure) Single ($\frac{1}{2}$) (heterozygous) Double ($\frac{1}{4}$) (sterile)

In these two crosses, *where the " singleness " in the F_1 (or later) heterozygotes comes entirely from the pure single parent,* we get what seems to be an ordinary Mendelian[3] result in F_2; the pollen of these heterozygotes must carry both "singleness" and "doubleness." The absence of singleness from the double-thrower pollen is taken by Bateson (1914, p. 292, foot-note) as almost conclusive evidence of somatic segregation of factors, occurring in such a way that the pollen-mother-cells receive only doubleness. Neither he nor Miss Saunders, however, gives any reason why singleness, rather than doubleness, should be thus eliminated. Goldschmidt (1913) and Belling (1915, p. 126) have stated that selective sterility of pollen will also explain the case, and definite evidence for this view is presented below.

[3] In some crosses the proportion of doubles is smaller, possibly 1/16 instead of 1/4.

To explain the slight but constant excess of doubles over singles, Miss Saunders assumes that two complementary linked factors, X and Y, are essential to singleness, and that these factors cannot be carried by the male gametes of the double-throwers, which are all xy. X and Y are supposed to be so linked in the ovules that the four kinds of eggs are produced, not in equal numbers, but in the ratio 7XY : 1Xy : 1xY : 7xy—or else in the ratio 15 : 1 : 1 : 15. Fertilization by xy pollen will give, in the former case, $7XY \cdot xy + 1Xy \cdot xy + 1xY \cdot xy + 7xy \cdot xy$; if only zygotes having both X and Y are single-flowering, only the first class will consist of singles, and the doubles will constitute 9/16, or $56\frac{1}{4}$ per cent., of the total. Linkage on the 15 : 1 plan would give 17/32, or $53\frac{1}{8}$ per cent., of doubles.[4]

For certain cases where crosses with pure singles have given much less than 25 per cent. of doubles in F_2, Miss Saunders assumes the presence of a second set of two linked factors, X′ and Y′; then any zygote receiving X or X′ together with Y or Y′ is a single, and the proportion of doubles is correspondingly reduced.

Von Tschermak (1912) favors Miss Saunders's hypothesis; he suggests the possibility of selective elimination (in a dihybrid scheme), but does not consider this explanation probable. It would seem, however, in view of considerations stated below, that any dihybrid scheme to explain the usual slight deviation of the double-throwing races from a 1 : 1 ratio is unnecessarily complex.

Goldschmidt's (1913) hypothesis assumes selective degeneration or sterility of pollen in the double-throwers, and considers the case to be one of sex-linkage, classing the slightly aberrant ratio with the known cases of slight deviation in the sex-ratio in animals. He supposes that this "hermaphroditic" plant is homozygous for a distinct factor for femaleness (F), producing eggs all of which carry this factor. He assumes that singleness is

[4] Miss Saunders (1911) rather favors the latter gametic ratio, which also corresponds closely to my own data.

determined by one dominant factor, S; the eggs of the double-thrower, then, are SF ("single") and sF ("double"). He assumes, also, that half the pollen-grains or microspores, in all races of *Matthiola,* lack F, probably because of elimination of part of an X-chromosome, and *that these pollen-grains degenerate* or at least are non-functional, so that no staminate plants are produced. It is necessary to assume, then, that in the double-throwing races S (or s) and F are carried by the same chromosome, and that the S-carrying chromosome is always the one to eliminate F. The S-carrying chromosomes will then be the ones destined to degenerate. The pollen resulting is of two kinds, Sf ("single," non-functional), and sF ("double," functional).

The double-throwing plant, then, is SFsF; its eggs are SF and sF, while its pollen-grains are Sf (non-functional) and sF. Self-pollination gives, then, SFsF (heterozygous or double-throwing singles) and sFsF (homozygous sterile doubles).

The factor S, however, can not in itself, in general, insure pollen-degeneration, since homozygous singles (SS) produce fertile pollen. Nor can the case be one of degeneration of all pollen-grains receiving *a maternal X-chromosome,* as is proved by the results of crossing SS and Ss races. Heterozygous singles (Ss) which get the S factor from a pure single (SS) parent, either through egg or through sperm, produce good S pollen, as is shown by the ordinary Mendelian ratio among their progeny (1 homozygous single (SS) : 2 heterozygous singles (Ss) : 1 homozygous double (ss). Goldschmidt is driven to assume, therefore, that the singleness factor (S_1) in the double-throwers differs from that in the pure singles (S)—or else to suppose that another factor interferes in the former type.

It will be seen that Goldschmidt gives, at most, only an indefinite implied explanation of the deviation of the double-single ratio from equality in the double-throwing races. And it is hard to see what advantage is secured

by introducing sex-factors into the discussion at all, when all actual individuals have both stamens and pistils, or else neither. When we assume—as Goldschmidt does—that the factor S is so modified in the double-throwing races as to insure the sterility of pollen-grains receiving it, the known facts must follow; it seems wholly superfluous to refer the sterility to linkage of S_1 with a sex-factor. The hypothesis seems quite unnecessarily complex; there is no real evidence here for the existence of a distinctly heritable femaleness factor, or for any elimination of sex-factors in pollen-formation, or for the occurrence of non-functional pollen in ordinary pure single (SS) races.

In a reply to Goldschmidt, Miss Saunders (1913) gives a very clear presentation of both her formulation and that of Professor Goldschmidt, urging most of the objections to the latter scheme which are stated above, but especially emphasizing its failure to explain the excess of doubles over 50 per cent. She also objects to the assumption of the existence of non-functional pollen, but I can not agree with her on this point.

I have sectioned anthers prepared for cytological study, and have frequently observed stages subsequent to the reduction divisions. The spore-tetrads appear normal, and there seems to be no early and conspicuous evidence of later degeneration. The "single" pollen, however, might even germinate and yet be strictly non-functional because of weak growth; and, as is shown below, the singles are actually inferior to the doubles in vigor. Selective partial sterility seems to be a rather common phenomenon, and it very probably occurs here.

The only other recourse seems to be the hypothesis of somatic segregation mentioned above, and somatic segregation, except as a rare accident of abnormal cell-division, has no decisive evidence in its favor[5] and an overwhelming convergence of probabilities against it. Belling (1915) calls attention to decisive evidence against

[5] Bateson's (1914, p. 292) positiveness in its favor seems to depend on just such cases as that here in question.

it in five genera representing as many distinct orders. Bateson himself (1909, chap. 9) reports a fact which seems to exclude it in the sweet pea, *although his reduplication hypothesis*[6] (Bateson and Punnett, 1911) *would require it there if anywhere.*

This phenomenon is one to which East (1915, p. 87) has recently referred, the "zygotic" nature of certain pollen-grain characters. In the sweet pea, for instance, F_1 hybrids between certain races with long (dominant) and round pollen have the pollen *all long,* although segregation, on any hypothesis, must have already occurred before the shaping of the pollen-grains. If segregation takes place as a result of chromosome-reduction, in the formation of the spore-tetrads, it is not strange that the cytoplasm of the young pollen-grain still retains the impress of the diploid maternal set of chromosomes, so that the pollen-grains give no evidence in their shape of the segregation that has just taken place. On the other hand, if segregation takes place early enough to permit of extensive "reduplications" of the cells carrying certain combinations of factors, it is very strange that the cytoplasm of the pollen-grain should be essentially maternal in nature. Especially does this evidence negative any hypothesis of cytoplasmic segregation—and if segregation is nuclear, surely we have reasons enough for connecting it with the reduction of the chromosomes.

It is due to Goldschmidt's hypothesis to note that a factor "completely coupled" with S, completely lethal for pollen and only slightly so for the embryo-sac, would explain the peculiarities of the case in *Matthiola,* both the non-functioning of the S-carrying pollen and the excess of doubles over 50 per cent. This is an amplification of his suggestion, in a passing reference (1913, p. 81), of a

6 Bateson and Punnett explain linkage of genetic factors by means of the hypothesis of somatic segregation. They assume that a period of cell-division intervenes between the segregation of Mendelian factors and the formation of the germ-cells, and that the cells bearing certain sets of factors divide more often than the rest. This would result in making some classes of germ-cells more numerous than others.

possible "further distinct hereditary factor"; I merely omit the sex-factor, and suppose the other factor to be lethal in itself. He evidently does not notice that this sort of factor might well explain more than the sterility of the pollen. It amounts to the same thing, however, to suppose the double-thrower S (or S_1) to be itself the lethal factor. The introduction of sex-factors seems entirely unnecessary here, and the supposed lethal elimination of an F factor can not be general in hermaphroditic plants, since it would involve the universal occurrence of sterile microspores or pollen.

Our case appears to be merely one of a hybrid showing selective sterility of pollen-grains, a sterility due to the S factor or to a lethal factor linked with S. Further, if there is also a slight tendency to selective elimination of S-carrying eggs, we have a simple and direct explanation of the excess of doubles over the expected 50 per cent. Or, if the s-carrying eggs are more often fertilized, the excess of doubles is explained. Once more, selective elimination of single (Ss) embryos might produce the same result.

There are several facts which are extremely suggestive in relation to all these possible forms of selective elimination. First, it is known that, in a double-throwing race, the doubles are longer-lived than the singles in the seed stage; Miss Saunders (1911, p. 362) has definitely confirmed the common belief that the proportion of doubles tends to increase with the age of the seed. Second, Miss Saunders (1911, p. 364) has obtained a higher proportion of doubles from seed of lower viability, even with fresh seed. Third, some seed-growers (deVries, 1906, p. 335) regularly "starve" the seed-bearing plants, in the belief that they thus increase the percentage of doubles among the progeny. Fourth, the writer (Frost, 1911) has found, with one variety, that inhibition of flowering by high temperature is much more marked with singles than with doubles; in field cultures, in many cases, hot weather greatly delayed or entirely prevented flowering, the

difference there being very much greater than that shown in the tables in the paper cited.[7] Fifth, in the cultures just mentioned the doubles had larger leaves than the singles and evidently were decidedly larger as young plants. It seems that the double form (ss) is superior to the heterozygous single (Ss) of this double-throwing race in general vegetative vigor, and a similar difference may exist between s and S gametes; *on these facts probably depend the peculiarities of the observed ratio.*

In order to make the case of doubleness in *Matthiola* as clear as possible, let us consider a brief summary of the formulations that have been proposed. There are two essential points to be explained, namely: (1) the fact that the singleness factor or set of factors of the double-throwing races can not be carried by functional pollen, although the corresponding factor or factor-group of the pure single races so far tested is normal in relation to pollen, even in single-double hybrids; (2) the fact that the double-throwing races show a small but fairly constant excess of doubles over 50 per cent.

Miss Saunders gives a formally adequate but rather complex factorial hypothesis for (2). She leaves (1), however, essentially unexplained; she evidently relegates it to the realm of somatic segregation, and in any case makes no suggestion as to the real cause of the *uniform* elimination of *singleness.*

Goldschmidt, on the other hand, gives for (1) a hypothesis of selective sterility which is adequate, though of obviously unnecessary complexity, but fails with (2) about as completely as Miss Saunders does with (1).

It is here maintained that an extension of the general idea of selective elimination or viability, in any one of the several forms consistent with the evidence, complies with all the requirements, adequately explaining both (1) and (2). It might seem, at first thought, that the assumption of a difference between S and S_1, or of the exist-

[7] This evidence is to be published mainly in my forthcoming paper on "Mutation in *Matthiola.*"

ence of a distinct linked lethal factor, makes this scheme as complex as that of Miss Saunders; this is not the case, however, since Miss Saunders's scheme simply omits any attempt at real explanation of the peculiarity of the double-thrower pollen; her formulation imperatively requires the addition of the hypothesis of selective viability, or of some definite equivalent for it.[8]

The real puzzle of the case lies in the fact that the double-throwers plainly differ from the pure singles so far tested in at least two respects—heterozygosity for singleness (ability to form sporophylls) and the association of some peculiarity with the remaining singleness. This, however, is essentially a problem of the *origin* of the double-throwing races, and is, in any case, nowhere simpler than with the hypothesis here suggested. Miss Saunders's scheme really implies four factorial or linkage differences between pure singles and double-throwing singles, and for certain cases six such differences, in place of the two or three required by the hypothesis here favored. That is, the double-thrower is supposed to differ from the pure single in the following points: (1) that it is heterozygous for two complementary factors (X and Y) for which the pure single is pure, and in some cases also for a second set of such factors (X′ and Y′); (2) that its "singleness" can not be carried by functional pollen; (3) that X and Y are partially instead of completely linked. It is here proposed to drop half the factors of (1), and this makes (3) superfluous.

[8] She supposes (Saunders, 1911, p. 334) that X and Y are completely linked in the pure singles, but only partially so in the double-throwers; this explains why, with self-pollination, the latter give 50 + per cent. of doubles rather than 50 per cent., *but not why they approximate 50 per cent. instead of 25 per cent.* It would seem, however, that the Ss hybrid between pure single (SS) and double-thrower (S_1s) may usually give double progeny approximating, not the 25 per cent. assumed, but a *slightly lower ratio.* A possible general slight deficiency of doubles in this cross is not provided for in Miss Saunders's hypothesis, complete linkage of X and Y explaining why there is not an *excess* of doubles; whether the viability-hypothesis is adequate depends on the general viability-relations of the S factor (as distinguished from S_1), which quite possibly is even superior to s in respect to vigor.

It must be admitted, however, that (3) is not in itself improbable if (1) is true, in view of Miss Saunders's evidence. A similar difference between races, with respect to linkage, occurs with "cream" flower-color, which is partially linked with doubleness in the sulfur-white races, but completely linked in the pure-cream races. The essential difference of the viability-hypothesis, as here presented, relates to (1); the demonstrated lower viability of the singles, evidently the basis of (2), makes possible the simplification of (1).

If we accept this viability-hypothesis, there seem to be two general possibilities as to the origin of the double-throwing races. One is that the mutation by which Ss (double-throwing) races arise from SS (pure single) races involves a simultaneous or consequent alteration in the remaining S factor (or the production of a lethal factor completely linked with S), by which the presence of S becomes incompatible with pollen-formation. Second, it may be that the particular race or races in which our double-throwing forms originated had an S factor originally different from that of the pure single races which have been used in crossing with double-throwers—that is to say, an S factor originally incompatible with the formation of good pollen in an Ss plant—or else that they originally possessed the lethal factor suggested. If the second supposition is correct, such pure single races may be found,—races which in crossing with double-throwers *never give the* F_2 *ratio 3 singles:1 double, but only approximately 1 single:1 double.*

With *Petunia*, if we ignore the new seed-producing double (Francis, 1913), which has a distinct type of flower, the general case would seem to be similarly simple. Here, as is well known, the doubles are produced only when singles are pollinated by doubles, the ordinary doubles having stamens but not pistils, or, at most, non-functional rudiments of pistils. In this case the doubleness factor (D) is plainly dominant, and is perhaps to be considered an inhibitor; the single, then, is

dd, and the double Dd, cross-pollination giving 1dd:1Dd. There is usually (Saunders, 1910) an excess of *singles;* here, as in *Matthiola,* the heterozygous form is the one deficient in numbers, and it is also the one which appears inferior in vegetative vigor.[9] Probably the deviation from the 1:1 ratio is due in *Petunia* to selective elimination of *doubleness.*

We have, then, in *Matthiola* and *Petunia,* hybrids evidently due, not to the crossing of widely different forms, but to mutation within the race,[10] and yet they are partially sterile, and perhaps even lacking in vegetative vigor because of their hybridity! In connection with the vigorous discussion of mutation now going on, it seems worth while to ask whether, in a case like that of *Œnothera,* hybridization is the cause of mutation or mutation one great cause of hybridity; apparently both views may be in part correct.

Miss Saunders favors a dihybrid scheme for *Petunia,* evidently supposing the difference here also to depend on two complementary factors, both necessary for singleness. Her assumption that singleness is dominant, as in *Matthiola,* seems absolutely untenable. In considering the last point, we may ignore the dihybrid feature, since this evidently concerns only the deviation of the ratio from 50 per cent.

Her formulation, as thus simplified, makes the singles Ss and the doubles necessarily ss; the data then indicate that the functional single pollen is all S-carrying (the reverse of the case in *Matthiola*), since self-pollinated singles produce no doubles. Then, either the single eggs are S + s, and the double pollen s + s, or the single eggs are s + s, and the double pollen S + s. The latter assumption is obviously impossible, since it not only contradicts the assumption that singleness is dominant, but

9 Theodore Payne, a seedsman of Los Angeles, California, says in his 1914 seed-catalogue, "The weaker seedlings should be carefully saved, as these invariably produce the double flowers."

10 This is not to assume that some disturbance due to crossing of two single-flowering forms might not have led to the "mutation."

makes both singles and doubles heterozygous (Ss);[11] the former assumption, however, is also excluded, as Miss Saunders shows, by the fact that all singles tested produce some doubles when pollinated by doubles—that is, the expected class of pure singles (SS) does not occur. Evidently, as both Goldschmidt (1913) and Belling (1915) assume,[12] *doubleness* is dominant in *Petunia,* and selective viability probably completes the explanation.

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11 In the dihybrid scheme, here, if linkage is to be invoked, as in *Matthiola,* to explain the deviation from 50 per cent. of doubles, both the singles and the doubles must carry *both factors*—since the singles possess both by hypothesis, and we are supposing the pollen of the doubles to show linkage! Further, doubles, not singles, would be expected to be in excess of 50 per cent. In fact, no self-consistent dihibrid scheme seems to be possible with *Petunia.*

12 Goldschmidt's statement (1913, p. 84) seems to suggest that Miss Saunders herself made this change, especially as he refers to "*Journal of Genetics,* **1**, 1911"; apparently, however, he intends the original article, published in 1910, and I have failed to find any reference to the matter in the part of the volume published in 1911. The explanation given above was stated in a letter sent to her in May, 1914, but no reply has been received. Bateson (1913) considers singleness to be dominant, admitting the necessary conclusion that all cultivated singles appear to be heterozygous. How this universal heterozygosity could be maintained in self-pollination of the singles (S + s eggs × S pollen, since the opposite assumption is untenable), he does not explain.

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